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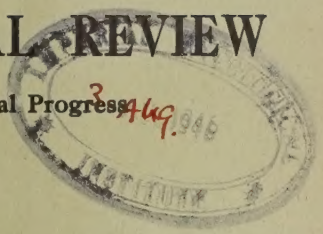

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THE BOTANICAL REVIEW

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PHYSIOLOGY OF CITRUS FRUITS IN STORAGE

E. V. MILLER[1]

U. S. Bureau of Plant Industry

Although there are many species and many hybrids in the group of plants which produce our citrus fruits, only five species enter sufficiently into commerce to merit consideration in a treatise on the physiology of citrus fruits in storage. These five are: (a) the sweet orange (*Citrus sinensis* (L.) Osbeck); (b) mandarin and tangerine oranges (*Citrus reticulata* Blanco), *i.e.*, Satsuma, tangerine, King, Temple; (c) grapefruit (*Citrus paradisi* MacFad.); (d) lemons (*Citrus limon* (L.) Burm. f.); and (e) limes (*Citrus aurantifolia* (Christm.) Swingle). Mention should be made here of some of the more common commercial varieties grown in the United States of America because, as will be shown later, the variety may have a direct bearing upon its subsequent behavior in cold storage. The leading varieties of early orange in the United States are: Parson Brown, Hamlin, Washington Navel. Midseason varieties are: Pineapple, Jaffa. Most of the seedlings are also harvested along with the midseason budded varieties. The leading late variety is Valencia. Typical seedy varieties of grapefruit are Walters and Duncan. The Marsh (seedless or nearly so) is believed to have originated as a seedling. The pink-fleshed varieties of grapefruit are bud mutations of some of the pallid varieties. The Foster (pink-fleshed) was developed from the Walters and is a seedy type. There are also two popular seedless pink varieties. One of these, the Thompson (Pink Marsh), is a bud mutant of the Marsh; and the Ruby is a bud mutant of the Thompson. Two varieties of lemon, Eureka and Lisbon, are grown commercially in the United States. There are, likewise, two common varieties of lime. The small, pale yellow, Key or Mexican lime was probably introduced into the United States by the early Spanish settlers. The Tahiti (Persian)

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lime is much larger, almost the size of the ordinary lemon, and is marketed in the green stage.

Citrus fruits are not held in cold storage to the same extent as are deciduous fruits. The latter must be removed from the tree before they become overripe. Citrus fruits, on the other hand, are usually "stored" on the tree until marketed. However, there are several exceptions to this statement. For example, when oranges and grapefruit are delivered to the metropolitan areas in quantities too large for immediate consumption they have to be held in storage. The Texas-grown grapefruit crop must be completely harvested before an established date (about May 1) in order to prevent infestation by the Mexican fruit fly. Adequate facilities for cold storage would help to extend the marketing period over the next two months. Another example of the use of cold storage facilities for holding citrus fruits is to be found in the lemon industry in California. The peak of lemon harvest in that state occurs during the late winter and early spring months, whereas the greatest demand for these fruits is usually in the summer. This may often necessitate a three- to six-month period of storage. Furthermore, the consumer demands medium-sized lemons which frequently necessitates picking and storing before they become too large. Other instances in which citrus fruits are subjected to at least the conditions of cold storage are the rail and steamship transportation of these fruits where the time of transit is sufficiently long to require refrigeration.

There is reason to believe that citrus fruits might be held in cold storage much more extensively in certain sections of the United States if facilities were available. In the Gulf States, for example, the commercial season for shipping oranges usually terminates about June 1. While it is true that up to this time oranges are held on the trees until marketed, many types of orange begin to "dry out" and regreen with the advent of warm weather, and could therefore be held much more advantageously in cold storage. This would serve to extend the marketing season well into the summer months because it has already been demonstrated by experiments in Florida that Valencia oranges may be stored until the latter part of July with 100% of the fruits remaining marketable (52). That there are problems that limit more extensive cold storage of citrus fruits is attested by the great amount of research being conducted on this subject in various parts of the world.

CHANGES OCCURRING DURING RIPENING

While this paper is concerned primarily with the physiology of citrus fruits in storage, it will be necessary occasionally to discuss certain phases of pre-storage physiology. The behavior of citrus fruits in storage may be greatly affected by certain cultural practices or by the manner in which fruits are handled after harvest. Likewise, since early and midseason oranges are usually "stored on the tree", so to speak, until a convenient time to ship them, it seems desirable to discuss briefly some phases of the physiology of citrus fruits during ripening.

Harding, Winston and Fisher (24) have described the changes occurring during the maturing of oranges on the tree and have presented a rather comprehensive review of the literature on the subject. Briefly these changes are as follows: There occurs a gradual increase in total soluble solids (principally sugars) and a decrease in acids, during ripening of oranges. At a certain stage of maturity the sugars and acids attain a proper balance and impart to the fruit a pleasantly tart or a sweet taste, and the fruit is considered ripe. Ascorbic acid (vitamin C), when expressed as milligrams per milliliter of juice, shows a slight decrease during ripening, this being more pronounced with Valencia oranges (late) than with early and midseason varieties. However, since ripening is accompanied by an increase in juice volume, the vitamin C is actually being diluted, and the loss is more apparent than real. Expressed on the basis of milligrams of ascorbic acid per individual fruit, there occurs an increase with ripening. Citrus fruits do not undergo a softening during ripening, as is characteristic of apple, peach, pear and banana. Other changes accompanying maturation are increases in size and weight, and a transition from green to yellow color in the rind. The changes in the constituents of the juice are very gradual when compared with those of deciduous fruits, so that the oranges may be held on the tree a considerable time before becoming overripe. In this connection Harding *et al.* state that: "Oranges left on the tree remain in good eating condition for a protracted period; however, if left too long granulation or drying out of the flesh, and an aged flavor, indicating senescence, eventually develop". Data presented by these authors indicated that early and midseason oranges left on the trees were considered by tasters to remain in the range of "pleasantly tart" to "sweet" or "very sweet" for two or three

months, and there was at least one plot where the fruit was acceptable to the tasters for over six months during the cool part of the year. It should be borne in mind that citrus fruits do not ripen after removal from the tree. These fruits do not contain a "carbohydrate reserve", like the apple, pear or banana, which is converted into sugar upon ripening. All oranges are therefore "tree-ripened" if they are ripened at all.

Physiological changes during ripening of grapefruit, lemons and limes are very similar to those occurring in oranges.[2]

CHANGES OCCURRING IN CITRUS FRUITS AFTER HARVEST

The object of storing fresh fruits and vegetables at relatively low temperatures is quite obviously the retarding of the processes which cause these foods either to decay or to become less attractive, less palatable and less nutritious. The changes which occur rather rapidly in unrefrigerated citrus fruits may be classified into three groups: (*a*) parasitic or microbiological; (*b*) physical; and (*c*) chemical. Under the term "parasitic or microbiological" are included the several types of decay which may cause considerable loss in citrus fruits after they are harvested. The most common of these are blue mold and green mold, caused by *Penicillium italicum* and *P. digitatum,* the stem-end decays (*Phomopsis citri* and *Diplodia natalensis*), and the core rots, caused by species of *Alternaria.*

The most obvious physical change occurring in citrus fruits following harvest is the loss of water. This results in wilting or shriveling of the fruit with accompanying decrease in weight and volume. This type of change affects only the appearance of the fruit and renders it generally unattractive, although it may be accompanied by chemical or physiological changes which cause a loss of flavor and aroma.

Among the chemical changes occurring in citrus fruits after harvest are losses in sugars and acids, which are consumed during respiration. A reduction in these constituents causes the orange or grapefruit to become less palatable. Certain changes in the pectic compounds are responsible for the development of a dry or "mealy" consistency. The reduction in ascorbic acid directly affects the nutritive value of the fruit, although this loss in itself does not affect

[2] Harding, P. L. and Fisher, D. F. Seasonal changes in Florida Grapefruit. U. S. Dept. Agr., Tech. Bul. 886. 1945.

the flavor of the fruit. The loss of aroma, on the other hand, is due to chemical changes which are so minute that they are difficult to determine by chemical analysis.

CHANGES OCCURRING IN CITRUS FRUITS IN COLD STORAGE

That low temperatures are effective in prolonging the life of fresh fruits is quite obvious from the extensive use of cold storage facilities in this country. That lowering the storage temperature retards but does not always stop some of the changes is not always apparent, and the fact that certain processes may continue uninterruptedly even in cold storage is often known only to the research worker. Furthermore, at low temperatures certain disorders may become pronounced that do not occur in fruit held at higher temperatures.

The operator of a cold storage plant does not have to worry about the decay-producing fungi in citrus fruits for these organisms are fairly well arrested or retarded in their growth by low temperatures. After about a month at 50° F. a certain amount of blue mold, stem-end decay, or *Alternaria* core rot may develop, but the percentage of decay is not usually commercially significant. When citrus fruits are removed from cold storage, the decay-producing fungi resume their activity, unless a disinfectant treatment has been applied prior to storage. The longer the fruits are held in cold storage, the sooner they will decay following removal from storage.[3]

Physical changes, such as loss of weight and loss of water, are liable to continue in cold storage unless precautionary measures are employed. These changes are pronounced if the relative humidity of the storage atmosphere is too low and especially when there occurs a rapid movement of air of low relative humidity in storage. A relative humidity of 85% to 90% is usually recommended for the storage rooms in which citrus fruits are held, although a satu-

[3] Detailed discussion of the use of disinfectants for controlling decay in citrus fruits is not included in this paper because such a discussion seems to belong to the field of pathology. Mention has been made of the borax treatment which in the past has been almost universally employed in packing houses, but which in recent years is being largely abandoned because of its cumbersomeness. Of all the many new disinfectants that have been tested in Florida for control of decay in citrus and which are apparently free from hazards to health, the diphenyl compounds show the most promise and are perhaps the only ones that are equal to borax in effectiveness. These diphenyl compounds can be applied in an aqueous bath, they may be incorporated in a wax emulsion, or may be introduced into paper wrappers or box liners. Thiourea and several similar compounds have not been included in this discussion of disinfectants because their status has not yet been established.

rated atmosphere would be ideal from the standpoint of preventing water loss in the fruit. Since a saturated atmosphere is conducive to growth of molds in the storage rooms, it cannot be employed, and the commercial practice is to maintain as high a relative humidity as is practicable. The packer of citrus fruits also usually takes the additional precaution of lightly coating the fruits with wax to retard water loss.

Changes in chemical constituents of citrus fruits are a little more involved than physical changes and have been the subject of more extensive investigation. Some of these studies were begun as early as 1920. Hawkins and Magness (30, 31) reported that grapefruit showed a slight decrease in acids during a storage period of four months at 32° F. There were no appreciable changes in sugar. The loss in acids reported was rather slight, rarely amounting to more than 0.2 in percentage value. Somewhat later Stahl and Camp (52) verified these results insofar as acids are concerned, but claimed that reducing, hydrolyzable, and total sugars increased during storage. These results are a little difficult to understand, since mature citrus fruits contain no appreciable amount of starch that could be converted into sugar, as is true of some other fruits (apple, banana, *etc.*). It is possible, of course, that certain cellwall materials, such as pectins and hemicelluloses, could be hydrolyzed into free-reducing substances.

The bitter flavor in immature grapefruit is caused by naringin, a glucoside. Similar glucosides are present in other citrus fruits but not to the same extent as in grapefruit. It is interesting to note that several of the early investigators (30, 31) reported that the naringin content of Florida grapefruit decreased when the fruit was held in cold storage for four months. The claim was made that cold storage improved the flavor of grapefruit by causing a reduction of both acids and naringin.

Harvey and Rygg (25) have made a rather extensive study of the changes occurring in grapefruit rinds in an effort to throw light on the physiological disorders which manifest themselves in the rind tissues. In these studies samples of grapefruit were collected from the Corona and Fontana districts in California, where the harvest follows a relatively cool period, and from a district near Oasis, in the Coachella Valley, where the grapefruit crop is harvested following an extremely hot and dry summer. The fruits were stored for six

weeks at 32°, 42° and 52° F. According to the results of these investigators, during the storage period there occurred a decrease in invert sugar in the rinds of the fruit from all sources. Reducing sugars increased at 42° and 52°, but the results for the 32° storage were inconsistent. Hydrogen-ion concentration increased at all temperatures and in both types of fruit.

There was a marked difference in the behavior of the fruit from the two areas in regard to changes in some of the other constituents of the rinds. Fruit from the Corona-Fontana district (harvest following a cool period) showed an increase in hydrolzable polysaccharides and a decrease in total soluble solids during storage. There were no great changes in these constituents in the fruit from the Oasis area. In the Corona-Fontana districts the naringin content of the rinds decreased at all temperatures, the greatest decrease occurring at 32° F. In the fruits from Oasis area, on the other hand, the naringin content increased at all temperatures, and the greatest increase took place at 32°. Hawkins, who reported a decrease in naringin content of Florida grapefruit in storage, collected the fruit from July 29 to December 9, but the fruits in which he reported changes in naringin content were collected during the cooler months (October, November and December). The fruits collected during July, August and September were not held at the lower temperatures. There is a strong suggestion in the reports from both California and Florida that naringin will decrease in stored grapefruit if harvested following relatively cool weather. However, Hawkins was referring to the juice, whereas Harvey and Rygg analyzed the grapefruit rind.

Additional contributions to the physiology of grapefruit in storage have been made by Rygg and Harvey (49). Samples of Marsh Seedless grapefruit were collected from three widely separated groves in California and stored at various temperatures. Total pectic substances in the albedo[4] showed a minimum early in the season for one lot and at midseason for the other two lots of fruit. The authors reported that variations of the total pectic substances in the albedo coincided with susceptibility to storage pitting, low pectic substances occurring at the time of low susceptibility. However, no causal relationship was postulated by the authors. Nar-

[4] "Albedo" is the accepted term used for the spongy, white layer of tissue just beneath the thin, pigmented "flavedo" of the rind.

ingin content of the albedo of grapefruit from the field varied in much the same way as total pectic substances.

Oranges are like grapefruit in regard to changes in quality that occur in cold storage. It is the general consensus among investigators (52, 69), for instance, that early and midseason oranges undergo a slight loss of acids and show no significant changes in soluble solids during cold storage. This has been reported for oranges stored for two weeks to two months. Similar results have been reported for Valencia (late) oranges with, perhaps, one exception. Stahl and Camp (52) reported a slight increase in hydrolyzable and total sugars in Valencia oranges stored for four months. These workers also noted an increase in specific gravity, pH value, and total solids in the juice, and a decrease in per cent of juice in the fruit. The claim has been made for "Common" oranges in New South Wales (Australia) (14) that palatability of the juice and texture and color of the rind were improved by cold storage.

As in grapefruit Harvey and Rygg (26) have conducted studies of changes in the rinds of oranges in storage. Analyses were made on Washington Navel oranges, which are harvested during the winter months in California, and on Valencia oranges, the season of which begins in May and extends through the summer. Valencia oranges were stored at 33° and 53°, while the Navels were stored at 32°, 42° and 52° F. The period of storage was approximately seven weeks. According to these investigators the differences in results can be attributed more to differences in season than to differences in variety. However, despite the apparent varietal or seasonal differences there were several instances in which the behavior in storage was similar for the two varieties. Soluble solids in the rinds of both varieties decreased in storage, the rate of decrease being greater at the higher temperatures. There was an increase in hydrogen-ion concentration during storage at all temperatures. Determinations of the glucoside hesperidin were made on the rind of the Navel orange only, and it was found that this constituent also increased in storage.

Of considerable interest are the analyses conducted separately of the stem-end and blossom-end portions of the orange rinds. The investigators state that in the Washington Navel orange "the stem-end showed greater changes than the blossom-end in all substances under observation except active acidity". They likewise reported

that in Valencia orange rinds the stem-end portion of the rind lost water and soluble solids more rapidly and contained higher hydrogen-ion concentration than did the blossom-end. The explanation of this increased physiological activity of the stem-end portion of the orange rind was to be found in the greater quantity of "albedo" tissue in this part of the fruit. The authors had previously reported the "albedo" tissue to be generally more "responsive" than the flavedo. These results are of considerable interest to the physiologist, and tend to confirm the observations of other investigators regarding differences between the two ends of the orange fruit. When Valencia oranges "regreen" in the spring, this renewed activity of the pigments always begins in the stem-end. Likewise "aging" and wilting in storage are always more pronounced at the stem-end.

In recent years considerable interest has been manifested in the vitamin C (ascorbic acid) content of oranges as well as the effect of prolonged storage on this constituent. According to reports from several sources, early, midseason and late oranges show no significant losses in ascorbic acid when held in storage for two weeks to three months. This has been reported by investigators in the United States (69), Australia (34) and in South Africa (23).

No great amount of work has been done on the storage of tangerines. Bratley (4) conducted some storage studies on the New York market in which these fruits were held for eight weeks at temperatures of 32°–33°, 36°–38°, 45°–48° and 53°–55° F. A marked loss of total acid and vitamin C was noted during storage at all temperatures, these losses being much greater at the higher temperatures.

The lemon, like the lime, has been used for both flavoring and therapeutic purposes for many years. It is of considerable economic importance, and the changes undergone by this fruit in storage should be of more than academic interest. Chemical analyses of California lemons stored at 32° to 60° F. give an idea of some of the changes occurring in these fruits in storage (43). Total sugar in both peel and pulp decreased in 15-weeks storage, the rate of decrease being greater at the higher temperatures. Analyses of the peel after 11 to 13 weeks showed increase in acidity and glucosides. Acetaldehyde tended to increase in storage, and this constituent was usually higher in fruit stored at 32°, 36° and 40° than at 50° or

60° F. Prolonged storage at 32° to 40° F. tended to reduce the reductase activity of the peel. In South African experiments (66) lemons were stored for four weeks at 40°, 45°, 50° and 55° F. When mature lemons still green in color were held at these temperatures, those at 50° F. developed more juice, soluble solids and acid than those held at the other temperatures. Studies made in California (27) indicate that during cold storage there is a reduction of the materials that are extractable by cold water or sulfurous acid. The compounds in question are no doubt those contained in the cell walls, such as pectins, hemicelluloses, *etc.* That there is no serious alteration in vitamin C content is indicated by the work of Delft (18) who stated that the antiscorbutic value of lemons remains unimpaired as long as the fruits remain in good condition.

RESPIRATION

Citrus fruits, like other fruits, continue to live for a while after removal from the tree. They respire, and during the process of respiration oxygen is absorbed, sugars and acids are oxidized, and water, carbon dioxide and heat are released. Generally speaking, the rate of respiration in citrus fruits is lower than in most other fruits and vegetables. It has been shown by several investigators (48, 22) that the rate of respiration of oranges at 32° F. falls within the range for that of apples and pears, which in turn are among the "low-respiring" deciduous fruits. The respiration rate for lemons and grapefruit is still lower than for oranges. When the temperature is raised, the rate of respiration increases much more rapidly in deciduous fruits than in citrus fruits.

In discussing respiration some mention should be made of the term "climacteric", which was first used by Kidd and West in connection with respiration of apples. After the setting of fruit, the respiration rate of various apple varieties is very high. The rate declines to a minimum at about the time most apple varieties are harvested, and then begins to rise again to a maximum. This peak in respiratory rate is termed the "climacteric". The respiration rate in apples and pears declines after the climacteric, and the fruits are said to be in senescence. A climacteric in respiration of oranges has been reported and will be discussed later (12).

A rather unique method for determining respiration has been developed by Harvey and Rygg (28, 49), and the method was found

useful in predicting the storage life of grapefruit, lemons and oranges. The fruits were stored in a glass jar to which a manometer was attached. Shortly after the fruits were introduced into the jar a negative pressure was produced which reached a maximum when the supply of oxygen was exhausted. With continued carbon dioxide production the pressure soon increased at a fairly constant rate, eventually becoming positive again. A relationship was found to exist between the negative pressure and the potential storage life of the fruits. Longer duration and greater maximum of negative pressure indicated longer potential storage life of oranges, lemons and grapefruit. Also the longer the duration of negative pressure, the less susceptible were grapefruits to pitting in storage. The authors point out, however, that "while the relationship of the time of negative pressure to susceptibility to subsequent pitting holds roughly for fruit from an individual grove or group of trees, it does not hold when the behavior of fruit from one location is compared with that from another, perhaps many miles distant."

Accumulation of carbon dioxide in storage may have a direct effect upon the physiology of citrus fruits. It requires but a slight percentage of carbon dioxide in the coloring rooms to retard the rate of degreening of citrus fruits. In cold storage a certain amount of this gas may prolong the life of the fruit, but too high a percentage will have deleterious effects. This is discussed later.

PHYSIOLOGICAL DISORDERS OCCURRING IN COLD STORAGE

Thus far only slight mention has been made of the difficulties frequently encountered in attempting to hold citrus fruits in cold storage for extended periods. These fruits, like other subtropical fruits, are rather sensitive to low temperatures, with the result that sooner or later they may develop certain physiological disorders. These disorders may not only render the fruits unsightly and unpalatable, but also make them very susceptible to decay upon removal from cold storage. The great danger zone for development of these maladies is in the range of 32° to 40° F. The upper limit should be raised a little for lemons and limes. Before entering into the discussion of the occurrence of these low-temperature disorders, a description of some of the more common injuries will first be presented. These descriptions are based on information appearing in the U. S. Dept. Agr., Miscellaneous Publication No. 498 (47).

Pitting (*pox, chill spotting, brown spot, storage spot*). Pitting of citrus fruits consists of abruptly sunken spots in the rind. The spots, though not discolored at first, may later become slightly pink on grapefruit and eventually brown on both grapefruit and oranges. Early and midseason varieties of orange are more susceptible to pitting than are late-ripening varieties, and the Pineapple orange is the most susceptible variety. Although pitting may occur at the time of packing or in transit, it generally does not develop until after a storage period of four to six weeks. Softening often occurs under the pits and may lead to invasion by blue mold and other fungi. The pulp underneath usually has a tainted taste.

Pits on lemons usually appear as depressed areas in the rind from 1/16 to 1/2 inch in diameter, sometimes retaining their normal color but more often becoming light brown or dark brown, approaching black.

A similar type of rind breakdown in limes is not referred to as pitting because large areas are usually involved, more irregular in shape. These areas are sunken, having distinct and abrupt margins, and they vary in color from tan through rusty pink to deep brown. Although affected areas may occur on any part of the fruits, they are more common on the sides than at the stem and stylar ends. The flavor of the juice is not affected unless the fruits are severely spotted.

Watery breakdown. Citrus fruits affected with watery breakdown look very much like fruit that has been frozen. They are soft and spongy and have a water-soaked appearance. Both flesh and rind may be softened. The carpels are loosely attached to the inner part of the rind and, when a section of the rind is pressed, a watery substance oozes freely from the albedo.

Brown stain or scald of oranges. Brown stain or scald of oranges consists of a superficial and fairly uniform browning over relatively large areas of the rind, differing from pitting in the large areas affected and in not being sharply depressed. Also the color is seldom so dark as that of pitting.

Aging. Aging, sometimes found on oranges and grapefruit, becomes apparent after harvest. The rind around the stem button or upper part of the fruit becomes wilted or shriveled, with or without collapse of the rind tissues. Sometimes it is difficult to distinguish between pits and aged spots on grapefruit and oranges. It is cus-

tomary in the United States to designate as aging any darkened and depressed spots in the rind in the vicinity of the stem.

Membranous stain or membranosis of lemons. Membranous stain of lemons is characterized by a browning or darkening of the membranes or carpellary walls between segments, sometimes affecting the central core tissues and inner tissues of the rind. There are no external symptoms. The irregular stained patches, varying in size from mere dots or streaks to large areas involving an entire membrane, are dark, yellowish-brown to sepia. This disorder is most easily detected by cutting the fruit lengthwise from button to nipple. The stain is seen on the divisions or membranes and not in the pulp cells or juice sacs. The juice is not perceptibly affected till membranous spots have occupied a large percentage of the membranes.

Albedo browning of lemons. Albedo browning takes the form of a discoloration of the white, spongy, inner part of the rind which is known as the albedo. The disease may be evident externally only as a slight darkening of the rind due to the discolored inner tissue showing through the surface layer. When the fruit is cut into, what is usually the white inner albedo portion is brown and sometimes shows a sort of gummy consistency.

Red blotch and peteca of lemons. There are several other rind blemishes of lemons which may occur in storage, although the fruits are first predisposed to these disorders by certain seasonal and weather conditions. Red blotch (adustiosis) is a superficial, scald-like browning of the surface layers of the rind. At first a light cinnamon brown, it may later darken into a chestnut brown.

Peteca resembles pitting, although the depressions are more gently rounded at the edges. The collapse begins with the albedo, and the outer layer of the rind sinks without first becoming discolored. The oil glands soon begin to darken, and in extreme cases the whole surface layer may collapse and become discolored.

Rind breakdown of oranges. Since pitting and aging of oranges are sometimes indistinguishable and since they are often both produced by the same storage condition, it is convenient to use the term "rind breakdown" to include both of these disorders in a discussion.

DEVELOPMENT OF PHYSIOLOGICAL DISORDERS IN COLD STORAGE

In studies with Florida grapefruit it was discovered as early as 1920 that the appearance of a breakdown or pitting of the rind was

an undesirable result of holding this fruit too long in cold storage (30). The range of temperatures in which this disorder was found most liable to occur was 31° to 42° F., although it occured to some extent at temperatures as high as 58°. Instances have been cited by other workers (52) in which Silver Cluster (seedy) and Marsh Seedless grapefruit, stored for eight weeks at 32° and 37½°, have developed so much pitting as to render 35% to 40% of the fruit unmarketable. The effect of low temperature on pitting of grapefruit has been reported from Florida, Texas and California in the United States, and from Trinidad, Palestine, South Africa and Australia (20, 25, 34, 38, 52, 61, 63). As a rule, temperatures around 40° have been found to be much more conducive to pitting than higher or lower temperatures.

Watery breakdown, another low temperature injury, has also been reported on grapefruit stored at temperatures ranging from 31° to 42° F. However it is more often reported to occur on fruits held at 32° or lower and it is believed by some that in instances in which this disorder has been reported to occur at 32°, the temperature may have accidentally dropped below this for a short period. The disorder does not occur except after prolonged storage. Watery breakdown gives the fruit the appearance of having been frozen, and it is possible that claims for damage have been paid on fruits, in storage or transit, affected with watery breakdown because both parties involved believed the damage to be due to freezing. It will be shown later that there are several other factors that predispose citrus fruits to watery breakdown in cold storage.

Because of the danger of low temperature injuries to citrus fruits in cold storage, investigators for many years have been seeking the optimum temperature for storing these fruits. The extent of this search for an optimum temperature is obvious from the fact that reports contain the following temperatures or range of temperature at which grapefruit have been stored experimentally: 31°–32°, 35°, 37°, 37½°, 39°, 40°, 42°, 45°, 48°, 50°, 52°, 54°, 55°, 58°, 60°, 70°–80° and 86° F. The choice of an optimum temperature involves of necessity a compromise between the prevention of decay which occurs at relatively high temperatures and the prevention of physiological disorders occurring at low temperatures. Citrus fruits produced under irrigation in areas of insufficient rainfall (California, Texas, Spain, Italy, Palestine, Australia, all but the

coastal areas of South Africa) are not so susceptible to stem-end decays as are the fruits grown in the regions having ample rainfall (Florida, South American countries, Japan, China, New Zealand). This probably accounts for the fact that investigators reporting on grapefruit from semi-arid regions designate 45°–55° as the optimum range of storage, whereas those in humid regions stipulate 32° to 37½°. The U. S. Department of Agriculture has taken this into consideration in its recommendations for the storage of grapefruit (48), which are: 45° to 55° for those produced in the sections of the country where stem-end decay is not a serious factor in holding fruit; and 32° for those grown in regions where this decay may shorten the storage life of citrus fruits.

The effect of low temperatures on stored oranges has been studied by research workers in many countries, and reports are available on these fruits produced in Australia, Brazil, India, Palestine, South Africa, Trinidad and the United States (2, 15, 34, 36, 38, 51, 52, 57, 59, 67, 75). The majority of these investigators have at one time or another reported the occurrence of low temperature injury, such as pitting of oranges when the fruits have been held for a month or more at temperatures below 40° F. In general, 36° to 40° has been found to be more conducive to pitting than higher or lower temperatures. In at least one instance oranges were stored in the range of 29°–32°, although this is much lower than is usually encountered in commercial storage houses. When the fruits have been held at or about 32° brown stain (scald) and watery breakdown have developed (26). In other words, a lower temperature is required to produce these two disorders than to produce pitting.

There has been at least one instance in which low temperatures are reported to have produced a bitter flavor in Navel oranges (75), in addition to the customary rind injuries, and there has been observed a type of spotting on this variety which appears at relatively high temperatures (50°–65° F.) and which differs from the usual low temperature breakdown of the rind (19, 64).

There is little agreement among investigators as to the optimum temperature for storing oranges. Taken the world over, the recommendations extend from 32° to 50° F. All of the investigators quoted have found one temperature or a very narrow range of temperatures that is more conducive to injury than others, but there

has been no complete agreement on the critical temperature or range of temperatures. As with grapefruit, the temperature selected for storage of oranges must be a compromise between an effort to prevent decay at high temperatures and loss from rind injury at low temperatures. For long storage of oranges the U. S. Department of Agriculture has recommended 34° to 38° F. (48). Recommendation from other sources have varied somewhat from this. For shipments from Brazil 36° F. has been recommended (11), and for shipment from California (45) to the Orient, 36°–38° F. State workers in Florida recommend 37½° F. and those in California, 38°–40° F. In Texas 50° F. is recommended for Valencia oranges. General recommendations from South Africa and Australia seem to extend into the higher range *i.e.,* 37° to 50° F. Van der Plank *et al.* (in South Africa) (64) state that temperatures in the region of 50° to 55° F. are indicated for oranges that are under-colored, hard-textured or otherwise immature and that are resistant to decay, whereas lower temperatures (such as 40° F.) are more advantageous for fruits that are fully ripe or overripe or that are greatly subject to decay. The fact that most of the oranges grown in South Africa are produced in the irrigated section and are thus less liable to decay, may explain the tendency of investigators in this part of the world to lean toward the higher temperatures in their recommendation for storage of oranges. From the results of experiments conducted by the U. S. Department of Agriculture the ideal temperature range for commercial storage of oranges appears to be 34° to 38° F.

Physiological disorders occurring in lemons at low temperatures have been reported in the United States (9) and in South Africa (66). When held at 32° to 40° F. these fruits have developed injuries such as pitting, watery breakdown, scald, red blotch and membranous stain. As with other fruits, scald and watery breakdown occur more frequently at 32°. Pitting is usually more pronounced at 32° and 36°, and red blotch at 36° and 40°. Membranous stain is much worse on lemons stored at 40° than at higher or lower temperatures. Lemons are much more sensitive to low temperature injuries than are grapefruit and oranges, and, as a consequence, higher storage temperatures are usually recommended for them, *i.e.,* 55° to 58° This, of course, increases the danger of loss by decay although this is usually the lesser evil in areas where

lemons are extensively produced. It has been found that green lemons stored at 50°–55° F. will develop as full color as those picked full-colored and stored at 40° or even better color (66).

Both Key and Tahiti limes are subject to rind breakdown in storage and transit (47, 68). These fruits are more subject to rind injury when held at 40° than at 45°, but if held long enough (eight weeks) at the higher temperature, they may still develop this disorder. Although this low temperature injury appears like pitting at first, it soon affects such large areas that it seems more like a distinct type of breakdown characteristic of only the limes. The damage is more severe on immature fruits than on mature ones, and limes stored in a dry atmosphere are more subject to breakdown than those held under moist conditions. Satisfactory storage of limes is usually obtained by holding at 45° to 48° F. with a relative humidity of 85% (48).

FACTORS AFFECTING THE INCIDENCE OF LOW TEMPERATURE INJURIES

Although the physiological disorders just described are usually referred to as low temperature injuries, there are certain other factors which, though not directly responsible for the injuries, certainly exert an influence on the fruits' susceptibility to low temperature breakdown. This may account in part at least for the great diversity in results that have been reported by research workers in different regions, not only in this country but in all parts of the world. Soil type, climate, cultural practices and position of the fruit on the tree are known to influence the subsequent storage life of citrus fruits. It has been observed in Florida that oranges and grapefruit produced in "low hammock" soils, which contain abundant moisture and are relatively high in organic matter, are more liable to breakdown in cold storage than are fruits grown on the "high pine lands" (39). Fruits produced in regions of abundant rainfall have a shorter storage life than those grown in semi-arid regions. Severity of pitting of grapefruit in storage has been shown to vary directly with the mean temperature for the five days preceding picking (49). It has been reported that grapefruit from trees receiving a high percentage of potash in the fertilizer has shown more physiological breakdown in storage than that from unfertilized trees (20). In South Africa grapefruits picked from the

outside of the tree were found to be more susceptible to cold injury than those from the inside (65). Another factor in susceptibility to injury at low temperature is the variety of fruit involved. In the United States early and midseason oranges as a rule are more susceptible to pitting than the late ripening varieties, the Pineapple orange being the most susceptible variety. The Valencia orange is more susceptible to aging than to pitting. Similar results have been reported for grapefruit. A number of investigators have reported that seedy varieties may be more satisfactorily stored than the seedless ones (20, 31, 52).

Still another factor that may influence the life of citrus fruits in storage is the stage of maturity at the time of storing. It is physiological maturity rather than legal maturity that is meant here. An orange is legally mature when the ratio of solids to acids in the juice has reached a certain value, this value having been set by the officials of the State in which the fruit has been produced. By physiological maturity is meant the age of the fruit with reference to its respiratory climacteric. This is illustrated by experiments in Australia (12) in which oranges were picked a month before the normal time of picking, at the normal time and a month later. Measurement of the carbon dioxide output (at 40° F.) of all pickings during the whole storage period showed that the fruit from the first picking reached its climacteric in 60 days; the second in 30 days; and the third had just reached its climacteric at the time of picking. The time to reach 10% decay in each pick was 70 days after the climacteric. Thus the storage life of the three picks was 130, 100 and 70 days, respectively, and the life of the fruit in cold storage was prolonged by storing it as much in advance of the climacteric as possible, although the last date of holding would be the same for all lots. In this connection Stahl and Camp (52) state: "from the standpoint of metabolic changes, it is best to store slightly under-normal mature fruit . . .". They illustrate their point by presenting the results of storing Valencia oranges that were below normal, normal and above normal in maturity. At 32° storage it required six months for the first lot to undergo certain changes in acids and sugar that required only three months on the tree. The mature lots required four months to reach the same stage that was attained in two months on the tree, and the last lot underwent certain changes in two months that required one month on the tree.

There is evidence, on the other hand, that certain physiological disorders are more liable to appear in citrus fruits when they are stored during the stage of under-normal maturity. In experiments with Florida oranges Winston (74) harvested the fruit at three different stages, corresponding roughly to three stages of maturity, although the exact date of the respirational climacteric was not determined in these particular instances. He found that, in general, the amount of pitting, aging and wilting in Valencia oranges in storage decreased with each successive picking. Similar results have been obtained with Palestinian grapefruit stored in England (21). The early-picked fruits were found to be more susceptible to low temperature injury than fruits from midseason and late picks. It has been reported that membranous stain and red blotch are more liable to develop in stored green lemons than in fully colored ones (19, 66). It is possible that when the climacteric in citrus fruits occurs at low temperatures, there is a serious disturbance in the metabolic processes. Certain waste products may be formed more rapidly than they can be thrown off. The beneficial effects of prestorage treatments, such as "curing" or exposure to carbon dioxide, may be due to their accelerating effect upon certain processes before the fruits are subjected to low temperatures.

If other conditions were equal and it came to a choice between storing oranges and grapefruit from early or late pickings, it seems more desirable to choose the latter. Holding the fruit on the tree would save cold storage charges, and, for a while at least, the fruit would be improving in quality. Stahl and Camp have advised against storing fruit that is over-mature because "it does not hold up well and takes on storage tastes rapidly". It is true that there is an end to all good things, but if the grower or shipper is familiar with his fruit he should be able to determine when the end is approaching. Some varieties of orange and grapefruit will last longer on the tree than others, and the same variety grown on sour orange rootstock will keep its quality on the tree longer than when budded on rough lemon stock. Grapefruits, being more acid than most oranges, usually maintain their quality on the tree for a longer time.

Relative humidity of the storage atmosphere is definitely related to the incidence of rind breakdown in citrus fruits. As mentioned previously, a low relative humidity will accelerate the rate of development of low-temperature injury of oranges, grapefruit, limes

and lemons. Pitting of grapefruit has been reported to be much worse in atmospheres of low humidity (65%–75%) than in atmospheres of high humidity (85%–95%) (7). Likewise, when both oranges and grapefruit have been exposed to a dry atmosphere by storing over sulfuric acid, loss of weight, loss of firmness and increase in pitting have resulted (54).

Since the physiology of citrus fruits in storage may be dependent upon the amount of processing received, a little space will be devoted to a description of customary packing house procedures. Legally mature citrus fruits that are still green in color are first "degreened" with ethylene gas. In this process the field crates of fruit are stacked in the "coloring room" immediately after picking. A mixture of air and ethylene, with a temperature of 80° F. and a relative humidity of 90%, is circulated in the coloring room. This mixture contains one part of ethylene to about 50,000 parts of air, and the process is continued for 24 to 72 hours, depending upon the amount of green pigment (chlorophyll) present in the rind. Following the ethylene treatment the citrus fruits are dumped on a conveyor belt and run through the packing house machinery. In the first process, which consists of washing, the fruits are immersed in a soak tank containing a solution of alkaline detergent. They are then removed by a moving belt, soaped, and scrubbed by passing over rotating brushes made of palmetto fibres, while water is sprayed on them from above. Early in the season, in Florida and Texas when the fruits "degreen" with a yellow rather than an orange color, they may be given a dye treatment which enhances the final color. This is the "color-added" process and must not be confused with the ethylene or "degreening" treatment which is sometimes referred to as "coloring". "Color-added" fruit is that which has had a dye added to the rind by means of a spray or soak tank. The dye is a compound like that used in coloring butter, and all oranges so treated must be stamped "color-added". Usually the temperature of the color-added solution is maintained at from 90° to 130° F. The fruits are next dried by passing through blasts of hot air. In the commercial processing of citrus fruits it is the general custom to add a light coating of wax. This may be applied to the fruits by revolving brushes that have first picked up the wax from a solid bar, or by a hot wax vapor, or a water-wax emulsion, or the wax may be dissolved in an organic solvent and sprayed on in the form of a fine mist. The

waxy coating serves both to give the fruit a polish and to retard shrinking. After receiving the wax, the fruits are polished by passing them over rotating brushes made of a soft fibre like horse hair. The next step in the packing house consists of grading, sizing and packing, either with or without wrappers. In some packing houses the fruits are given an application of borax for the purpose of reducing decay. Oranges are dipped in a saturated solution of borax either on the loading platform prior to the ethylene treatment or in the packing house prior to waxing. The temperature of the borax bath varies with the packing house and may be as high as 100° F. It is followed by a rinsing with water.

The effect of packing house processes on rind breakdown and decay of citrus fruits in storage has been studied rather extensively by Winston and Roberts (72). Working with most commercial varieties of orange they collected samples of processed and unprocessed fruits from packing houses in various parts of Florida. These authors summarized their results as follows: "The washing, color-adding, ethylene and waxing treatments given oranges in packing houses increased rind breakdown considerably. All steps appeared to contribute to the increase. . . . Decay of gassed or color-added fruit decreased with increased processing, while the amount of decay in fruit which was not colored was unaffected by processing. Juice quality, judged by total soluble solids, total acid, and vitamin C content, was not generally affected by washing, dyeing or lightly waxing the fruit. In a few instances flavor was definitely impaired". Bratley and Winston (5) found the same results with fruits after they were shipped to the New York market.

METHODS FOR PREVENTING LOW-TEMPERATURE BREAKDOWN

It is not surprising that numerous attempts have been made by research workers to develop methods for preventing or reducing physiological disorders of citrus fruits in cold storage. One of the earliest methods tried was that of holding the fruit at a higher temperature for a brief period before placing in storage or transport. This process is referred to as "quailing", "curing" or "wilting". Success with the method was first reported by Hawkins and Magness (30) in their work with grapefruit, and as a result of subsequent experiments (31, 32) recommendations were made for curing grapefruit for one to two weeks at 70° to 75° F. with a

relative humidity of 65%. Similar results were subsequently reported from Texas (20). Brooks and McColloch (7) found a curing period of three to five days at 60° satisfactory, but 50° F. was not satisfactory. These authors also controlled pitting in grapefruit by heating to 100° F. for 17 to 22 hours before storing at 36° or 40° F., but this treatment increased scald and produced increased amounts of pitting when the fruit was subsequently stored at 32° F. In South Africa (62) it was found that there was an optimum period of delay for grapefruit and that prolonged delayed storage was worse than none at all. The optimum found was one to two days at 80° F. or four to six days at 65° F. Attempting to place curing on a quantitative basis, other workers in South Africa (16) reported a reduction in pitting if the grapefruit was first cured to a point where it had lost between 3.3% and 5.0% of the original weight. In Trinidad (37) it was reported that while a certain amount of wilting is desirable during the rainy season, it can be eliminated during the dry season. However, curing of grapefruit has not been adopted as a regular practice in the industry.

Curing or quailing of oranges prior to cold storage has not been found particularly advantageous (46, 56).

Attempts have been made to control low temperature injuries to citrus fruits by adding a protective cover to the fruits, either by applying a coating of oil, wax or water-wax emulsion directly to the surface of the fruits, or by enclosing the fruits individually in wrappers. Among the many types of wrappers tried are those made of tissue paper and known as apple or dry citrus wraps, or the same type of wrappers impregnated with mineral oil, paraffin or a mixture of both oil and paraffin. There are also moistureproof or semi-moistureproof wrappers such as wet-waxed paper, parchment paper, cellophane, pliofilm and aluminum foil. Control of pitting in grapefruit has been obtained by the use of a coating consisting of a mixture of mineral oil and paraffin, but this tended to increase scald and watery breakdown if the fruits were held at 32° F. (7). Beneficial results in controlling pitting of grapefruit have been obtained by wrapping with waxed paper (20), cellophane or aluminum foil (53). Some control of rind breakdown of oranges has been reported by the use of moistureproof and semi-moistureproof cellophanes, pliofilm or aluminum foil (53, 55, 70). The use of certain types of mineral oil in orange wrappers has increased rather than prevented injury.

CARBON DIOXIDE STORAGE

The use of carbon dioxide in the storage atmosphere should be mentioned here because this gas has been employed by research workers for the purpose of prolonging the storage life of citrus fruits by attempting either to prevent rind breakdown or to retard the incidence of senescence and decay. Nelson (44) first suggested that the composition of the storage atmosphere may have a direct bearing on physiological disorders in citrus fruits because he was able to produce "brown spot" (pitting) experimentally by storing oranges and grapefruit in pure nitrogen. He concluded that the disorder was caused by lack of oxygen. However, several years later Brooks and McColloch (7) decreased the amount of pitting in grapefruit by reducing the oxygen content of the atmosphere by adding carbon dioxide. Their treatment consisted of exposing the fruit to atmospheres of 20% to 45% carbon dioxide for 20 to 48 hours prior to removal to cold storage. Similar carbon dioxide treatments reduced both pitting and membranous stain in lemons (9). Stahl and Cain (54) also were able to reduce rind breakdown in citrus fruits through the use of carbon dioxide, and recommended storing these fruits in an atmosphere containing 6% of carbon dioxide and 12% of oxygen. In general, most attempts to store citrus fruits in an atmosphere of high carbon dioxide content have met with failure, the result being usually some type of injury to the rind, a deleterious effect on flavor or even an increase in fungal wastage. Results of this type have been reported by investigators in Australia, England, Palestine and Trinidad and by several in the United States (8, 14, 37, 58, 59). The periods of storage in these instances lasted from four weeks to three months, and the percentages of carbon dioxide have ranged from 5% to 81%. Although some success with relatively high percentages of carbon dioxide has been reported in England (1) the workers in the United States who have reported no injury to citrus fruits stored in atmospheres rich in carbon dioxide have either held the fruits for much shorter periods (about one week) or have employed lower concentrations of carbon dioxide (10% to 15%) (6, 8, 54, 58).

To a certain extent excessive dosages of carbon dioxide produce effects similar to those of prolonged storage. For instance, small amounts of acetaldehyde and alcohol are to be found in normal citrus fruits; but these substances increase with prolonged storage, especi-

ally at low temperatures (32° to 40° F.) and are likewise increased by treatment with high concentrations of carbon dioxide (14, 42, 43). It is possible, therefore, that the physiological disorders, previously described, are the result of auto-intoxication, caused by accumulation of the products of anaerobic respiration or of other abnormal enzymatic processes that have been induced by the environment.

ETHYLENE TREATMENT

One is tempted to give this chapter the title of "The Romance of Ethylene" because few chemical compounds have made so dramatic an entry into an industry or have continued to hold the interest of physiologists so long as has this unsaturated hydrocarbon gas called ethylene. This story begins with the beginning of the 20th century when it was customary to ship oranges to northern markets in railroad cars heated with kerosene stoves to prevent freezing. At that time it was noted that green fruits in the car assumed the characteristic orange color during transit, but this was considered to be merely a ripening process due to the heat generated by the stoves. However, in 1912 Sievers and True (50) showed that the active coloring agent was to be found in the products of incomplete combustion of the kerosene and that the heat generated was of secondary importance. Furthermore, the fruit could be "degreened" by the exhaust gases of a gasoline motor. In 1924 Denny (17) demonstrated that the active ingredient in the kerosene fumes was an unsaturated hydrocarbon gas. This suggested ethylene, a well-known gas of this type, and, accordingly, a small amount of pure ethylene mixed with air was found to color citrus fruits very rapidly. Thus began the practice of coloring or "degreening" with cylinders of ethylene gas, which is described earlier in this paper. Citrus fruits that are customarily given the ethylene treatment are the early oranges, such as Washington Navel, Parson Brown and Satsumas, as well as fruits of any variety of orange or grapefruit that fail to color properly on the tree because of having grown in the shade of dense foilage. In addition, the Valencia (late) orange usually develops a good yellow color in the winter while still immature, and then starts turning green at the stem end as it matures in the spring. These fruits are given the ethylene treatment to remove the green pigment (chlorophyll) from the rind. There is still another instance in which ethylene may be used in the processing of citrus fruits.

Lemons are picked according to size and as a result there may be green fruits among the yellow ones when they reach the packing house. The green lemons are sorted out and colored by storing at 50° to 55° F. for 30 to 60 days before marketing, but if the demand is brisk, they are subjected to forced curing or coloring with ethylene.

Degreening with ethylene is usually considered a coloring process rather than a ripening of the fruit. That is, there is no effect upon solids, acids, *etc.* that would render the fruit more palatable (73) as is the case with fruits like the banana. On the other hand there are definite physiologic changes that result from the ethylene treatment. Denny (17) found that forced curing of lemons is accompanied by greatly increased respiration and the formation of an abscission layer resulting in the loss of the stem "buttons". Ethylene treatment definitely affects the storage life of citrus fruits in that it has been reported to increase the amount of pitting and aging in oranges (72) as well as red blotch, albedo browning, and to some extent membranous stain in lemons (19), and also to accelerate decay in oranges, tangerines, grapefruit, lemons and limes (71). In accelerating decay of citrus fruits ethylene may function either as a stimulant to the biological activity of decay-producing organisms, or may produce a weakening in the cell wall structure of the host. Brooks (10) states that under some conditions ethylene stimulates the growth of the fungus *Diplodia natalensis,* one of the causal agents in stem-end decay. Other workers have found that ethylene treatment of fruits causes changes in the pectic constituents, including the middle-lamella pectin (29, 33, 41).

Interest in ethylene was revived among physiologists by the discovery that this gas is evolved by fruits and vegetables as a product of normal metabolic activity. As early as 1910 Cousins (35) reported that gaseous emanations from oranges accelerated the ripening of bananas although he was apparently unaware of what gas was being evolved. Between 1933 and 1938 a number of investigators were reporting that ethylene or a gas similar to ethylene is evolved by living plant tissue. In 1940 Biale (3) and Miller, Winston and Fisher (40) reported independently that ethylene is evolved by citrus fruits and that the evolution of this gas is more rapid in decaying fruits than in normal ones. It was also shown in both investigations that the organism that produces green mold rot in citrus fruits (*Penicillium digitatum*) is likewise capable of pro-

ducing ethylene. It is beyond the scope of this paper to review the many peculiar physiologic effects that small quantities of ethylene can produce on fruits, flowers, tubers, *etc.*, in storage. Suffice it to say that the evolution of ethylene from ripening fruits in storage has often produced undesired effects on other commodities and has necessitated storing them in separate compartments. It is evident, therefore, that proper storage of citrus or other fruits requires some knowledge of living processes in plants.

SUMMARY

As citrus fruits mature there is an increase in size, weight and volume of juice, and a change from green to yellow color in the rind. There occurs a gradual increase in total soluble solids and a decrease in total acids. There occurs a slight decrease in milligrams of ascorbic acid per milliliter of juice, due to increase in juice volume, but this appears as an increase when expressed as milligrams per individual fruit. Citrus fruits are unlike some fruits in that they do not ripen after removal from the tree.

If held too long at relatively high temperatures, citrus fruits may be attacked by decay-producing organisms or they may undergo physical and chemical changes which render them unattractive or less palatable and less nutritious because of loss of solids, acids and other compounds which impart flavor and aroma to them. Retarding of these changes is accomplished by holding in cold or cool storage. Recommended temperatures for storage are: Grapefruit, 45° to 55° F. in regions where stem-end decay is not a factor, but 32° where liability to this decay may shorten the storage life; oranges, 34° to 38°; lemons, 55°–58°; and limes, 45°–48°. Thus, under proper conditions oranges may be stored for eight to ten weeks. grapefruit and limes for six to eight weeks, and lemons for one to four months without any significant losses in nutritional value. There have been reports indicating that cold storage has improved the flavor of grapefruit by causing a reduction in the quantity of the bitter principle known as naringin.

On the other hand, too long storage at certain temperatures may produce physiological disorders such as aging, brown stain or scald, pitting, watery breakdown, albedo browning, membranous stain, peteca and red blotch. Some of the factors that have been reported to predispose citrus fruits to these low-temperature injuries are a

high percentage of potash in the fertilizer, a relatively high content of moisture and organic matter in the soil, the susceptibility of specific varieties, harvesting fruit after relatively high mean temperatures, storing fruit from the outside branches of the tree or fruit that is physiologically immature, processing in the packing house and low relative humidity in the storage rooms. Methods reported by research workers for reducing the amount of physiological disorders in storage consist of delayed storage, application of waxes and wrappers to the fruits, and exposure to carbon dioxide.

Rate of respiration in citrus fruit is generally a little lower than that of most fruits and vegetables. However, citrus fruits are like some of the other fruits in that after maturity they attain a respirational "climacteric", after which they go into senescence. The onset of senescence in citrus fruits has been delayed by storing them prior to the incidence of the climacteric, and the life in cold storage thus extended, although some evidence has been reported suggesting that the development of the climacteric in storage accelerates the production of physiological disorders.

Prolonged storage of citrus fruits in relatively high percentages of carbon dioxide has usually resulted in rind injury or in a deleterious effect on flavor. Investigators reporting success with carbon dioxide storage of citrus fruits have employed moderate percentages of the gas (10% to 15%). There is some evidence that a very brief treatment with high percentages of carbon dioxide reduces the amount of some of the physiological disorders subsequently developing in cold storage, but on the whole the carbon dioxide storage of citrus fruits is still in the experimental stage.

Under certain circumstances ethylene gas is employed commercially for "coloring" or degreening citrus fruits. The treatment removes the green pigment (chlorophyll) from the rinds of the fruit but does not measurably affect the solids, acids and vitamin C in the juice. However, ethylene stimulates respiration, causes the stem buttons to be released, produces certain changes in pectic substances and appears to accelerate the development of rind breakdown and decay. The increase in decay in ethylene-treated fruit may be due either to chemical disintegration of cell-wall materials, to biological stimulation of the decay-producing organisms, or to both.

In recent years citrus fruits, like other fruits, have been shown to evolve ethylene as one of the products of normal metabolism, and

the evolution of this gas is more rapid in decaying fruits. The peculiar physiologic effects of ethylene on stored fruits, vegetables, flowers, *etc.*, has added to the problems of cold storage and indicates that a knowledge of the physiology of fruits and vegetables is essential to the successful storage of these products.

If a concise recommendation for storing citrus fruits is desired, it can be expressed as follows: Eliminate insofar as practicable the pre-storage conditions that have been described in this paper as undesirable. Select the temperature recommended for the particular type of fruit and attempt to maintain optimum conditions in storage. If the loss of moisture is being retarded by the use of moistureproof wrappers, inclusion of a suitable disinfectant in the wrapper is highly desirable. Make periodic inspections of the stored fruit in order to terminate the storage at the very first symptoms of development of physiological disorders.

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THE EFFECT OF MINERAL SUPPLY ON THE MINERAL CONCENTRATION AND NUTRITIONAL QUALITY OF PLANTS

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INTRODUCTION

The mineral composition of plants is a function of many factors, such as difference in soils, use of soil amendments or fertilizers, and rainfall and other climatic influences. These factors overlap in their effects or work simultaneously. One factor may influence another. Thus, the effect of climate on the plant is partly direct and partly the result of the development by climatic factors of certain soil characteristics. Differences in these factors will naturally operate to modify the mineral composition of the plant in different ways. For example, it is possible (*a*) for the mineral composition of two plants of the same variety growing in different soils to be significantly different without there being any important difference in their size or the distribution of their parts, such as leaf, stem or seed head; (*b*) for the growth (yield) of plants of the same variety to vary in different soils without any important differences in the proportions of the parts of the plants; (*c*) for two plants of the same variety growing in different soils to have quite different distributions of leaf, stem or head; and (*d*) for environmental factors to so modify the quantities of plant constituents such as protein, carbohydrate, lignin and cellulose as to influence the percentage distribution of other constituents, as by a deposition of starch with a consequent reduction in the percentage composition of the mineral elements. The properties of two soils may be such as to modify the natural flora and thus to produce plants quite different in mineral composition. The evaluation of the influence of these factors on plant composition is still proceeding, and much valuable information of fundamental importance is being obtained.

The nutritional diseases of animals which have been traced to soil characteristics may be divided into two general classes. The first,

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and geographically more important, are those due to a deficiency of one or more of the nutritionally important inorganic elements in the food that eventually result in bone maladies, anemias, goiter and related diseases. Diseases of the second class are the result of excesses of certain mineral elements in the soils. Of this class, selenium toxicity has been extensively studied.

Because of modern methods of food distribution, troubles in man due to mineral deficiencies in food are rarely traceable to any specific soil condition, although such disorders as goiter are especially prevalent in areas where water and plants are deficient in iodine, indicating a deficiency of available iodine in the soil. Nevertheless, the protective foods such as milk, eggs, fruit and vegetables should be maintained at optimum nutritional levels, for a diet supplying minimum quantities of these foods may be reduced below the level required for good nutrition if the quality is inferior. Although many factors other than poor quality of food probably are responsible for nutritional troubles in both man and animals, this factor is believed to be an important one.

Fertilizer experiments have for the most part been concerned with the study of yields, and the problem of modifying the composition of the plant has been much less often the principal objective. That the composition of the plant is modified by the use of fertilizers has been known since the earliest soil fertility studies were made, and, in fact, plant composition has frequently been used as a guide for determining the nutrient requirements of the soil. For the purpose of the discussion which follows, investigations of that nature will not be considered, but an attempt will be made to assemble and organize the data and conclusions from those experiments designed primarily to improve the composition of the plant from the nutritional point of view. This field of investigation is in a state of high fluidity; ideas and objectives have not been clearly defined in many cases, and results are consequently not as conclusive as might be desired. Only a very few experiments have been reported that deal with human foods. Those concerned with animal feeds have utilized the forage crops as experimental subjects. Reviews of the effects of fertilizers and soils on crops, particularly forage crops, covering the literature up to about 1940 have been published (13, 26, 105, 116). The following review has been limited for the most part to papers appearing since that time.

EFFECT OF FERTILIZATION ON THE PHOSPHORUS CONCENTRATION IN PLANTS

The possibility of increasing the phosphorus concentration in forages has its most practical aspect, of course, where the level of phosphorus in the plant is below that required by grazing animals. The minimum level necessary for animals may vary with conditions but is believed to lie somewhere between 0.13% and 0.18% on a dry-weight basis. These low levels are adequate only for maintenance of the animal; for growth and lactation, much higher values are generally accepted as being necessary. It is of particular interest, therefore, to examine those cases in which investigators have been able to produce by fertilization forages containing adequate supplies of phosphorus where under natural conditions the levels of this element in the forage were normally very low.

Effect of phosphates. Most experiments designed to test the effect of phosphate fertilizers in increasing the phosphorus concentration of plants growing on soils deficient in this element have given positive results even though limited in magnitude. Superphosphate is, of course, the usual source of phosphates. Applications of from 100 to 500 pounds per acre are common, and higher quantities have been used in experimental work. The absolute change in phosphorus content of the plant is small, generally much less than 0.10% on a dry-weight basis, even with the heaviest applications of phosphates. Moser (86), for example, in pot tests in which he applied superphosphate at the rate of 600 pounds per acre to a Cecil sandy loam, was able to increase the phosphorus level in Austrian winter peas and lespedeza from 0.16% or 0.17% to only slightly over 0.20%. Larger quantities of phosphates did not result in any greater increase of phosphorus concentration in these forages.

Similar results have been obtained in field tests by other investigators (18, 35, 49, 60, 72, 84, 115, 120) where superphosphate alone has been applied to carpet grass, Kentucky bluegrass, lespedeza, Austrian winter peas, common vetch, wheat, potatoes and sugar beets. It is important to note that in all these studies observations have been made on single species or on mixtures that have not been altered significantly as a result of fertilization. Changes in botanical composition as a result of fertilization are commonly experienced. In South Africa (54), for example, it was found that

the application of phosphates to a plot of a very deficient veld soil resulted in herbage containing 0.14% of phosphorus in one season and 0.17% in the next as compared with 0.07% in the herbage from an untreated plot. The phosphorus concentration, thus doubled, would probably supply the minimum amount within the range required in forage. However, in the original herbage *Tristochya hispida* and *Digitaria tricholaenoides* were the dominant species. After fertilization of the soil, *Digitaria tricholaenoides* increased in amount while some other species nearly disappeared. Phosphorus concentrations in the individual species are not available, but it may be presumed that a change in botanical composition might have contributed materially to the relatively large increase in phosphorus in the total herbage.

A relatively low content of phosphorus in herbage is not always a justification for expecting even a limited increase from fertilization with phosphates. In pot work with the Gila clay in New Mexico, Hinkle (56) found that the phosphorus concentration in clover was not significantly altered as a result of applications of phosphates, even though the original amount in the clover, 0.17%, was relatively low for this plant (13). In the field tests he obtained a significant increase in the phosphorus concentration in alfalfa, although the highest level he obtained was well below the average reported in the literature (13). Other investigators working with potato plants (69), alfalfa, oats and wheat hay (117) and cowpeas (7) found that applications of phosphates to the soil did not result in any significant increase of phosphorus in these plants. However, a number of investigators (7, 21, 25, 27, 48, 52, 53, 70, 75, 92, 96, 104, 117, 123, 124) have reported increases in the phosphorus concentration in forage and other crops even where the original phosphorus level was relatively high.

Application of phosphates to range lands is not always considered to be an economical procedure. In South Africa, where there is reported to be an increasing tendency to revert to stock farming in preference to grain production, it is felt that the necessary phosphorus can be supplied animals grazing phosphorus-deficient velds by supplemental feeds as rich as possible in this element. Theron (113) has accordingly studied the effect of phosphates on the phosphorus concentration in corn in this region of relatively low rainfall and dry-land cultivation practices. He reports that the

annual application of 100 pounds of superphosphate per acre resulted in increases of phosphorus equivalent to 7% to 28% of that of untreated grain. Large applications up to 400 pounds did not result in any further increases in the phosphorus concentration, although crop yields were further increased. It is interesting to note, however, that recent reports from range investigations of southern Texas (16) indicate that phosphate fertilization of range lands may be more practical than feeding the mineral directly to the animal.

Effect of nitrogen. Certain of the effects of nitrogen fertilization on plant growth, such as a greater vegetative development, might well be expected to influence materially the concentration of mineral constituents of the plant. This might be particularly true when rapid and unusual vegetative growth occurs on a soil where minimum quantities of mineral elements are available. The agronomic "luxury consumption" might be considered to be zero under these conditions if the rate of uptake of mineral elements by the plant did not increase with increase in yield. However, the problem is complicated by other reactions in the soil, many of which are not clearly understood. Certain nitrogenous fertilizers undergo changes in the soil that result in increasing its acidity, and as such they might be expected to render certain mineral elements more available. Conversely, some "antagonistic" effects are also apparent, such as the depressive effect of nitrogen on calcium absorption. The net effect of nitrogen will be, therefore, a function of many soil characteristics.

Some investigators (21, 56, 108) have noted that nitrogen fertilization resulted in increased phosphorus concentration in forages, while others found either no effect (27, 49, 52, 54, 117) or a depressive effect (19, 77, 115) on phosphorus concentration. Snider (108) reported, for example, that applications of either $NaNO_3$ or $(NH_4)_2SO_4$ to Kentucky bluegrass resulted in doubling the phosphorus concentration, but even his high value, 0.16%, is below the average for this species (13). Blaser and Stokes (19) noted a reduction in the phosphorus concentration of carpet grass from 0.16% to 0.14% when 72 pounds of nitrogen per acre were applied to Bladen fine sand. Variable results were obtained on Leon soil, but the phosphorus concentration of the grass grown on that soil was well above the minimum required for grazing animals. The magnitude of increase in yield was similar for both soils.

Lorenz (72) found that the omission of nitrogen from the fertilizer mixture resulted in as low a concentration of phosphorus in the leaf blade of the potato plant as did the omission of phosphate. The highest phosphorus concentration was obtained with the highest application of nitrogen. Knowles, Watkin and Cowie (69), however, reported that nitrogen applications did not modify the phosphorus concentration of the potato plant as a whole under their conditions. Other investigators have reported only slight or no effect on phosphorus concentration of nitrogen fertilization alone on the wheat plant (53) or turnip greens (104), although in the latter case less phosphorus was found in the turnip when nitrogen was added with phosphate than when phosphate was added alone.

Effect of nitrogen with other fertilizer elements. Bledsoe and Sell (21) noted that the phosphorus content of Bermuda grass was higher when both nitrogen and phosphates without lime were applied to the soil than with either of these materials alone. Sheets *et al.* (104) found no significant difference in the phosphorus content of turnip greens as a result of applying nitrogen, phosphates and lime.

Effect of potassium fertilizers on phosphorus in the plant. There seems to be general agreement that the phosphorus content of the plant is either unchanged or lower where potassium has been supplied as a fertilizer than where it is omitted. Some investigators (19, 53, 69, 115) have noted significantly smaller concentrations of phosphorus in forages, potato plants and cereals, while others (20, 49, 54, 104) have reported no change associated with fertilizing with potassium. Leichsenring and Donelson (70) concluded from a statistical analysis of their data that potassium fertilization resulted in a lower phosphorus concentration of potato tubers, but the differences are very small and are of no practical importance. Blaser, Volk and Stokes (20) reported that the use of 1,500 pounds of limestone with 75 pounds of potassium chloride per acre had no effect on the phosphorus concentration of lespedeza.

Effect of potassium and phosphates. McClendon and Mayton (78) found under conditions in the Alabama Gulf Coast region significant increases in the phosphorus concentration in pasture herbage where potassium or limestone and potassium were added along with relatively high applications of phosphates. The level of phosphorus in the treated pastures was still low, but greatly in-

creasing the applications of fertilizer failed to result in any further change in composition of the forage. The authors do not report the botanical compositions of the treated and untreated pastures, so that it is not certain whether this is due to a change in composition of individual species or to a change in the relative amounts of the forage species present. Sheets *et al.* (104) found no significant change in the phosphorus concentration in turnip greens as a result of applications of potassium, limestone and phosphates. Increases in the phosphorus concentration in alfalfa and sweetclover have been reported by Davis and Turk in Michigan when these crops were fertilized with potassium and phosphorus (36). It is instructive to note, however, that the level of even unfertilized forages in Michigan is much higher than in the heavily fertilized forage in Alabama. McClendon and Mayton (78) have noted this predominant effect of location within different areas in Alabama. Thus, soils and climate under these conditions may have a far greater effect on plant composition than does soil treatment. Blaser, Volk and Stokes (20) found no change in the phosphorus content of lespedeza when 450 pounds of superphosphate and 75 pounds of potassium chloride per acre were applied to the soil. Sheets *et al.* (104) found a significant decrease in the phosphorus content of turnip greens when phosphorus and potassium were applied to a number of different soils.

Effect of liming materials. It is well known that when added to acid soils, limestone represses the solubility of iron and aluminum and converts the insoluble phosphorus compounds of these elements to more soluble forms. An excess of limestone, of course, will react with the phosphates to form insoluble basic compounds. The effects of limestone, therefore, depend upon the particular conditions existing in the soil as well as on any possible interactions with other elements. The most striking effects of limestone in relation to phosphorus seem to have been on yields rather than on the phosphorus concentration in the plant. Investigators working with forages (3, 20, 24, 49, 115) in many parts of the country have not noted any change in the phosphorus concentration as a result of applying lime, although in most cases yields were increased. Some others (19, 35, 62, 78, 118, 120) noted a lower phosphorus concentration in several forage species as a result of liming. Hoover (60) reported that the phosphorus concentration of vetch in the spring

was higher with liming than without but that later in the year this was not true. Fudge (50) found that applications of 400 pounds of limestone per acre resulted in a higher concentration of phosphorus in orange juice. Sheets *et al.* (104) noted no change in the phosphorus concentration in turnip greens grown on acid soils throughout the Southeast as a result of liming.

Effect of liming materials and phosphates. The reports of several investigators indicate that the combined application of limestone and superphosphate to the soil has more influence on the concentration of phosphorus in the plant than either of the materials alone. Davis and Brewer (35), for example, found that without limestone they were unable to obtain an increase in concentration of phosphorus in legumes with phosphates alone. Blaser, Volk and Stokes (20) have reported significant increases in the phosphorus concentration in lespedeza when limestone and superphosphate were applied, and others have noted increases in phosphorus in pasture herbage (46, 51, 78). Modification of botanical composition is a probable contributor to the change in the latter cases. Sheets *et al.* (104) found no significant effect of limestone and superphosphate on the phosphorus concentration in turnip greens.

Effect of nitrogen, phosphates and potassium as a complete fertilizer mixture. The foregoing reports on the effect of individual elements on phosphorus content indicate considerable variation. Studies of combinations of nitrogen, phosphates and potassium as mixed fertilizers do not offer any clarifying details with regard to these relationships. Lack of systems for sampling pastures and hays, disregard of species differences in the samples, and failure to measure normal variations are often important factors in this confusing situation. For example, Fink (48) has pointed out that a nearly threefold increase in the phosphorus content of fertilized native pasture herbage was largely due to the effect of a complete fertilizer in promoting the substitution of white clover and Kentucky bluegrass for poverty grass (*Danthonia spicata*). Other workers (27, 53, 74, 78, 101, 117, 121) also have reported obtaining much higher phosphorus contents in mixed pasture herbage as a result of fertilization with nitrogen, phosphates and potassium. As a result of application of a complete fertilizer, Vandecaveye (117) found a significant increase in the phosphorus of the first cutting of pasture herbage but not of the second cutting. He reported, however, no significant changes in the phosphorus concentration in

individual species, such as alfalfa and oats and wheat cut for forage. Other investigators also have not observed any effect of complete fertilizers on the concentration of phosphorus in wheat grain and straw (53) and turnip greens (104). Tyson (115) reported a definite increase in the phosphorus concentration in Kentucky bluegrass when a mixed fertilizer was applied to one soil, although the increase was not as great as when phosphates only were added. On another soil, however, less phosphorus was found in the bluegrass from plots receiving a complete mixture than in that from the check plots. All phosphorus concentrations were well below average (13). Alben and Hammar (1), studying the effect of fertilization of Orangeburg fine sandy loam on the composition of pecan leaves, noted only slight increases of phosphorus when complete fertilizer mixtures were used.

Effect of complete fertilizer mixtures and liming materials. Smith and Albrecht (107) and Guyon (53) have reported significant increases in the phosphorus concentration in mixed hays and in individual species as a result of fertilization with a complete fertilizer and lime. These results seem to be due largely to the addition of the lime in some experiments (53), while in others (19) the application of phosphates was most effective in producing a higher phosphorus content of the plant. Sheets *et al.* (104) did not find any significant effect of this treatment on the phosphorus content of turnip greens.

Effect of other elements. Nieschlag (90) reported slight increases in the phosphorus concentration in lupine, the potato plant and the rye plant as a result of applications of magnesium compounds to the soil. Fudge (50), working with oranges, did not detect any consistent effect of even very large applications of magnesium carbonate on the phosphorus concentration in the juice. Leichsenring and Donelson (70) observed no significant change in the phosphorus concentration in the potato tuber with applications of iron sulfate to the soil. Harmer and Benne (55) found that applications of sodium chloride to muck soils resulted in higher phosphorus concentrations in celery and sugar beets only where potassium was deficient.

EFFECT OF FERTILIZATION ON THE CALCIUM CONTENT OF PLANTS

Deficiencies of calcium have seldom been reported in cattle and sheep, and Russell (100) states that "there is no evidence that

grazing animals suffer from a straight acalcicosis"; but Maynard (83) points out that "generalizations regarding the adequacy of grass hay in calcium content for animal nutrition are unsafe because of the very large variations that can occur". A study of available data would indicate that most legumes will always supply sufficient calcium for animals. However, the possibility of a below-normal calcium concentration in plants and the great importance of this element in both human and animal nutrition cannot be denied. It is, therefore, of interest to examine the effects of fertilization on its concentration in the plant.

Effect of liming materials. It is recognized that liming practices tend to encourage the growth of species relatively high in calcium in both pasture and forages for hay. The growth of many of the legumes, such as white clover, soybeans, sweetclover, alfalfa and red clover, react markedly to limestone, rapidly displacing the nutritionally less desirable grasses and other plants. Since these legumes are higher in calcium than the species ordinarily constituting the native pastures, the calcium concentration in the forage as a whole is often greatly increased. While there is considerable evidence of this in the literature, there is less evidence of significant changes in the calcium concentration in individual species. Relatively small increases in calcium have been observed in carpet grass (19, 49), Austrian winter peas (*Pisum arvense*) (24, 86) and vetch (*Vicia sativa*) (24), although increased yields of these species were obtained as a result of liming. Differences in the response of species to liming have been observed by Vanderford (118). He found that the yield of and concentration of calcium in soybeans, Korean lespedeza and sweetclover were higher on limed plots but that the increase of calcium concentration was much greater in lespedeza than in the other species. Many investigators have reported no consistently higher concentration of calcium in Korean lespedeza, red top, sweetclover (3), vetch (26), corn stover, corn grain (120) or Kentucky bluegrass (3, 115) as a result of liming. In most instances, of course, yields were increased.

Calcium is of more interest in human nutrition than is phosphorus because of the greater possibility of deficiencies of this element in the diet. Consequently, the possibility of increasing the calcium concentration in plants grown for food has been considered by several investigators. Sheets *et al.* (104), in a very extensive in-

vestigation covering the soils and climatic conditions of a number of southern states, have concluded that the calcium concentration in turnip greens was not greatly affected by the calcium applied to the soil. Fudge (50), studying the composition of orange juice, reached similar conclusions, at least for normal applications of limestone. Some higher values were obtained (9.25 mg. per 100 ml. of juice as compared with 7.75 mg.) when an application of 3,200 pounds per acre of limestone was added to the soil. No consistent effect was noted on the calcium concentration in the foliage. Other workers have reported somewhat higher calcium levels in a number of vegetable crops (44), potato leaves (89) and alfalfa (62) as a result of increasing the calcium supply of the soil.

Effects of nitrogen. It has been recognized generally that application of nitrogenous manures to the soil is associated with a lower calcium concentration in the plant than is found under natural conditions. Recent findings confirm this. Ammonium sulfate as a nitrogen carrier seems to be more effective in this respect than sodium nitrate. Apparently, location, as expressed in terms of climate and soil, may modify these effects markedly. In Georgia (7), for example, the calcium concentration in cowpeas fertilized with nitrogen was lower than normal at Experiment but at Blairsville was unaffected by fertilization. Marked reduction in the calcium concentration in carpet grass (19) and Bermuda grass (21) has been noted, particularly where high applications of nitrogen were made. Effects of nitrogen on the calcium concentration in mixed forages and pasture herbage may be associated with changes in botanical composition as well as with changes in the chemical composition of individual species. Brown and Maunsell (27) found that a lower calcium concentration in pastures fertilized with nitrogen was accompanied by a greater prevalence of bluegrass and less clover. Other investigators (12, 42, 115) have observed this effect on the calcium concentration in pastures, but botanical analyses have not always been reported.

Knowles, Watkin and Cowie (69) and Lorenz (72) found that applications of nitrogen resulted in lower concentrations of calcium in the leaves of potato plants. Sheets *et al.* (104) reported that in all possible fertilizer combinations of nitrogen, potassium, phosphorus and calcium, nitrogen produced the most marked effect on calcium in turnip greens—that is, significantly lower values for

calcium were obtained in 24 out of 30 experiments in which nitrogen was applied as a fertilizer. Guyon (53) reported little effect of nitrogen on the calcium concentration in wheat grain or straw. Vandecaveye (117) noted little effect of nitrogen fertilization on the calcium concentration in several forages in Washington. Bledsoe and Sell (21) found that nitrogen and phosphate fertilization resulted in lower concentrations of calcium in forages. Even the addition of lime failed to establish a calcium content per unit weight equal to that in forages from unfertilized soils in Georgia.

Effect of phosphates. In field practice phosphorus is ordinarily applied to the soil as a calcium phosphate along with calcium sulfate (superphosphate). Consequently, the effect of phosphorus alone on the calcium content of plants can seldom be measured. In common with the results on the influence of limestone and other calcium compounds, many investigators have reported very slight or no change in the concentration of calcium in plants as a result of the use of superphosphate as a fertilizer (21, 53, 60, 70, 78, 84, 86, 92, 107, 117, 120). In a few instances, superphosphate has been associated with higher percentages of calcium in carpet grass on certain soils but not on others (19), while in the same region, Florida, it was noted that no change occurred as a result of the use of superphosphate if liming were ample (20). Significant increases in the calcium concentration in pastures without a change in plant population have been reported (115, 123) as a result of fertilization with superphosphate. Similar results have been found for the potato leaf (72). Sheets *et al.* (104) reported significantly less calcium concentration in turnip greens fertilized with superphosphate as compared with no superphosphate. Hirst and Greaves (57) have reported that the calcium concentration in sugar-beet leaves grown on soil fertilized with calcium sulfate was lower than in those from untreated plants. Much more information concerning the behavior of gypsum in superphosphate is needed to clarify the effects of this calcium carrier.

Effect of potassium. Investigators using a wide variety of species and soils are almost unanimous in their conclusions that applications of potassium result in a repressed absorption of calcium by the plant accompanied by a lower concentration of the element in most instances (23, 40, 45, 53, 57, 69, 72, 82, 102, 115, 120), although it has been observed that where no increase in yield oc-

curred, the calcium concentration in some forage species has not been affected materially (49, 115). Others (19, 20, 104, 117) also have observed no effect of potassium on calcium concentration, but relationship to yield is not always apparent. Hunter (61) reported a marked increase in the concentration of calcium in alfalfa as a result of increasing calcium supply and decreasing potassium supply in a Dutchess loam soil.

Effect of nitrogen, phosphates and potassium as a complete fertilizer mixture. The marked influence of soil and climate on the effects of fertilizer treatment is again demonstrated in the work in Georgia, where at Experiment (7) the calcium concentration in cowpeas was lower with application of complete fertilizers, while at Blairsville no difference between fertilized and unfertilized soils was noted. Lucas, Scarseth and Sieling (73) found that heavy applications of complete fertilizer mixtures resulted in decreases in calcium concentration in mixed hay, alsike clover and red clover. The calcium concentration in corn, wheat and soybean plants was not affected. Most reports (42, 53, 70, 78, 104, 117)are negative with respect to any marked effect of applications of complete fertilizers on the calcium concentration in plants, although yields were commonly increased as a result of their use.

Effect of complete fertilizers and liming materials. The effect of climate in modifying the influence of soil treatment is suggested by the work of Blaser and Stokes (18), who reported no significant change in the calcium concentration in carpet grass (*Axonopus affinis* Chase) in 1937 as a result of applications of complete fertilizer and limestone, although very significant increases were obtained in 1939 and 1940. Yields also were very much greater in the latter years. The authors do not attempt to explain this difference except to ascribe it to climatic conditions. Other investigators (73, 115) have noted increases in calcium concentration in some forages when both limestone and complete fertilizer were used, but not all crops responded equally. No response was noted in wheat grain or straw (73), timothy (60, 107) or turnip greens (104).

Effect of other elements on the calcium content of plants. There is a general agreement in recent work (28, 44, 45, 50, 90, 122) that applications of magnesium to the soil appear to reduce the calcium concentration in plants. The differences are often small and in most cases may be of no practical importance except where the

calcium level is normally low. For example, Willard and Smith (122) reported from 13% to 32% less calcium in various species grown on magnesium-fertilized plots, but only in timothy did the calcium concentration approach a level believed to be limiting with respect to nutritional value. Sulfur may have the effect of increasing the calcium absorption by the plant, according to the findings of Chapman and Brown (29) in experiments with orange trees.

EFFECT OF MICRONUTRIENTS ON THE MINERAL COMPOSITION OF PLANTS

Increased boron concentration in a number of plants has been demonstrated in several instances (33, 67, 88) where boron was added to the soil. The effect of boron supply on the utilization of other elements by the plant seems to be more variable. Boron had no effect on the calcium or phosphorus concentration in a number of forages in the experiments of Nowosad (91) and Cook and Millar (33). Jones and Scarseth (67) also reported little effect of boron on calcium concentration except at very high levels of boron supply, where yields were reduced. Under these conditions, the calcium concentration in alfalfa and oats was reduced. Applications of boron to the soil have been shown to have reduced markedly the iron concentration in sugar-beet roots and spinach leaves (71). Additions of limestone may reduce the concentration of boron in plants (67, 88), although applications of boron with limestone generally restored boron concentration to its original level in these experiments.

Teakle (114) has reported that application of from two and one-half to ten pounds of bluestone per acre to pasture land will raise the copper content of the herbage from 3 to 7 or 12 p.p.m. Johnson (66) has reported that sulfur had no effect on the copper concentration in alfalfa growing on a sulfur-deficient soil. MacIntire (80) found no increase of fluorine concentration in Sudan grass from applications of calcium fluoride to soils in pots.

There is little evidence that addition of iron compounds to the soil has resulted in increased iron concentration in plants. At least the differences that have been reported are variable and of little practical importance (70). Additional recent evidence that liming the soil would reduce the iron (93) and manganese (14, 27, 93, 110) content of plants under certain conditions has been obtained.

No effect of gypsum on the iron concentration in alfalfa (66) or turnip greens (110) has been observed. In an extensive factorial experiment, designed to test the effect of all combinations of nitrogen, phosphates, potassium and calcium on the concentration of iron in turnip greens (110), it was found that a significantly lower iron concentration was associated with nitrogen fertilization. Brown and Maunsell (27) found a lower iron content in pasture herbage where phosphates and potassium had been applied. When limestone was included in this treatment, no further effect on iron was noted.

The effects of liming materials on the micronutrients in forage have continued to receive attention. Albrecht and Smith (4) in pot tests found that mixing the limestone throughout the soil resulted in less absorption of manganese than occurred when the limestone was incorporated in the surface two or three inches only. Additions of sulfur (93) and ammonium sulfate (109) have been reported to increase manganese absorption. Johnson (66), however, did not observe any change in the manganese concentration in alfalfa growing on a sulfur-deficient soil fertilized with gypsum. It has been noted (4) that applications of phosphates were associated with increased manganese concentration in the plant.

Ferguson, Lewis and Watson (47) have found that use of limestone or other basic materials on pastures favors absorption of molybdenum by the herbage, whereas ammonium sulfate and other acidic materials have the opposite effect. Millikan (84) found little effect from ten pounds of zinc per acre on the calcium, phosphorus, zinc or manganese concentration in wheat plants. There was a slightly greater zinc concentration, however, where superphosphate had been applied. Harmer and Benne (55) could find little effect of sodium chloride on the calcium concentration in plants except with very high applications that depressed the absorption of calcium.

Additional work has confirmed the fact that the cobalt level of pasture herbage can be successfully increased by use of cobalt compounds (9, 111), of cobaltized superphosphate (8, 22, 68) or of limestones high in cobalt (38, 99). In areas where soils are low in cobalt, application of nitrogen to increase yields may reduce the concentration of cobalt in the plant (63), although where the supply is adequate this will not always occur (14).

INVESTIGATIONS WITH NUTRIENT SOLUTIONS

A limited amount of recent work in solution cultures has contributed materially to our knowledge of how mineral supply acts in modifying the mineral concentration in plants. In addition to observations that the quantity of calcium and phosphorus found in the plant are proportional to the supply in the nutrient solution, some effort has been made to study interactions. The contributions in the latter field have included studies with both major and minor elements.

Phosphorus. It has been observed that phosphorus concentration in the plant is depressed by a deficiency of magnesium in the culture solution (15). Phosphorus concentration seems to be inversely proportional to the supply of potassium in the nutrient solution in some cases (11, 34, 98), although Bartholomew (11) found that while this was true in the leaves of tomato, the reverse was true for the stems. McCalla and Woodford (77) observed that limiting nitrogen in the culture solution resulted in increased phosphorus concentration in the plant. It also appears that a high nitrogen supply may be responsible for depressed phosphorus content per unit of dry matter (34, 106). Scott (103) did not note any change in phosphorus in the plant where the ratio of potassium to sodium, and of potassium to magnesium, were increased in the nutrient solution throughout a considerable range. Richards and Sheng-Han (98) noted an increase in the phosphorus concentration in barley with increase of the sodium supply in the nutrient solution. Dickman and DeTurk (37) found that young corn plants grown in sub-irrigated gravel cultures and supplied only with phosphate rock as a source of phosphorus were limited in growth and phosphorus content as compared with those supplied a soluble source of phosphorus. In general, nutrient culture work is not a satisfactory guide for soil phosphorus studies because of the great differences in the level of phosphorus supply and the character of the phosphorus compounds in the soil as contrasted with the conditions in nutrient solutions.

The hydrogen ion concentration of the solution is of importance in the calcium-phosphorus interaction, according to Moser (87). At a low pH, an increase in calcium supply did not result in any change in the phosphorus concentration in soybeans, although at a pH of 6.0 to 6.5 there was an increase in phosphorus. There is

some evidence of species differences, however, for the phosphorus concentration in lespedeza increased with increase of calcium supply at all pH levels, while no changes occurred in the phosphorus content of sorghum. Chapman and Brown (30) found no differences in the phosphorus concentration in orange leaves from seedlings grown in nutrient solutions with either high or low calcium supply.

There are some indications that high iron supply in nutrient solutions may be associated with a decreased concentration of phosphorus in plants (106), but the differences are of little practical importance. Bartholomew (11) has pointed out that an abundant nitrogen supply actually increased the concentration of iron in plants in his experiments.

Calcium. Sideris, Young and Krauss (106) and Jacobson and Swanback (64) found that the concentration of calcium in plants was greater in the presence of nitrate ions than of ammonium ions in the nutrient solution. Calcium concentration in the plant tends to be inversely proportional to the supply of potassium in the nutrient solution (10, 15, 30, 112), although Scott (103) found no change in the calcium concentration in *Chlorella pyrenoidosa* with an increase in the ratio of potassium to sodium in his solution and a marked increase of calcium as he increased the ratio of potassium to magnesium over a wide range. It is generally agreed (10, 15, 71, 87, 112) that the calcium concentration in plants will increase with increase in the calcium supply of nutrient solutions. In other experiments (106), iron had no effect on the calcium concentration in the test plant. Barbier (10) reported that magnesium depressed the calcium concentration in a number of plants, but others (15), using the tomato plant, did not confirm this.

Studies with soybean (85, 119) and tobacco (112) have demonstrated that the calcium concentration in these plants grown in nutrient solution is roughly proportional to the boron supply when sub-optimal supplies of boron are present. When super-optimal supplies are present, this relationship becomes an inverse rather than a direct one (85). In general, when boron supply was optimum for growth, the calcium content of the leaf blades of the soybean was at a maximum. In the corn plant, however, boron supply did not greatly influence calcium concentration, although both boron and calcium concentrations in the plant were directly related to the supply of each element, respectively, in the nutrient solution (81).

Minarik and Shive (85) point out that the inconsistent results so far obtained as to the effect of boron supply on calcium metabolism suggest that if boron actually does influence calcium metabolism, its effect varies with the species of plant investigated and with the conditions under which the experimental procedure is carried out. Lorenz (71) found that an increase in boron supply in the nutrient solution resulted in increased growth of garden beets and increased absorption of calcium, but no increase in the content of calcium per unit of dry weight. He believes that boron may be more closely associated with the utilization than with the absorption of calcium. Reeve and Shive (97) conclude that calcium accumulation in the tissues of tomato plants is largely determined by calcium supply and appears to be independent of boron. Similar conclusions have been reported with the cotton plant (59).

Micronutrient elements. A high level of calcium supply has been associated with reduction in concentration of manganese and iron (112) in tobacco plants grown in nutrient solutions. Most investigators using nutrient solutions have shown that the concentration in the plants of micronutrient elements iron, molybdenum, boron, manganese, zinc and copper is dependent upon the level of supply available in the culture solution (6, 39, 59, 76, 81, 85, 106). Reeve and Shive (97) found that for any given boron supply in the substrate there is a progressive increase in the concentration of boron in the plant as the potassium supply increases. This was especially pronounced at high levels of boron supply. Chapman and Brown (30) found that when potassium was omitted from the nutrient solutions, peach tree leaves absorbed excessive quantities of boron. Reeve and Shive did not work with these low levels of potassium. Sideris, Young and Krauss (106) reported an inverse relationship between iron supply and manganese concentration in *Ananas comosus.* Chapman (31) found that excessive zinc supply in sand cultures resulted in a reduced iron concentration in lemon plants.

Others (94) have studied the effects of boron supply, ranging from deficient to toxic concentrations, on the chemical composition of tomato leaflets. The concentration of 14 elements was determined in the plant, and it was found that there were significant and large differences, depending upon the boron supply. The concentration of some elements was altered as much as several hundred per cent.

EFFECT OF FERTILIZATION ON THE QUALITY OF FORAGES AS MEASURED IN TERMS OF ANIMAL GROWTH AND HEALTH

It is not uncommon to measure the returns from pasture fertilization in terms of increase in production of animal products per acre. Increases in yield and the introduction of more nutritious forage species have been shown to improve the grazing value of land in terms of weight gains of animals or increase in days of grazing (5, 16, 17, 27, 41, 43, 51, 54). To test the effect of fertilization on the nutritive value of a single forage species is a more difficult matter and is not commonly done. Fundamentally, however, this is a question of considerable interest and importance.

Albrecht and his co-workers (2, 58, 79, 107) have attempted to measure improvement in the biological factors in forage plants resulting from the use of fertilizers, particularly calcium and phosphates. Their general approach has been to use animal growth as an index of the changes in crop quality in response to soil treatment. This is based on the justifiable assumption that the animal might respond to changes in the composition of the forage that are not detectable by ordinary chemical analyses. They found, for example, that lespedeza grown on soil treated with both phosphate and lime contained about 17% more protein than that from untreated soil. They conclude that it is not unreasonable to suppose that organic compounds other than proteins also may be influenced favorably.

In their experiment, carried on in the fall of 1939 (2), they report a 50% greater gain in growth of lambs fed lespedeza from soils treated with limestone and superphosphate as compared with that from untreated soils. Unfortunately, their experimental hay crop was partially destroyed by rain after harvest, and the portion recovered was supplemented by other hay grown under uncontrolled conditions. It is necessary, therefore, to consider very critically any conclusions in regard to the effect of soil treatment on crop quality in this experiment. This was recognized by these investigators, and the experiment was repeated the following year when conditions of harvest were more favorable. In this experiment, it was found that sheep fed lespedeza hay from limed soils gained 0.1644 pound per day as compared with 0.1408 pound for those on lespedeza from unlimed soils. The composition of the two hays was not signficantly different with respect to the constituents determined—nitrogen, calcium and phosphorus. The authors conclude,

therefore, that the animals responded to differences in forages not detected by chemical analyses.

A careful scrutiny of the data, however, reveals the following: (*a*) apparently no account was taken of possible variations in botanical composition; (*b*) no report was made as to the proportion of leaves to stems in the two hays; and (*c*) no attempts were made to determine the digestibility of the two hays. That these factors were probably of considerable importance is shown by the fact that about 50% of the hay from unlimed soils and 46% of the treated hay was refused by the animals. This is an unusually large waste in an experiment of this nature. Where wastage is of this order, it is manifestly impossible for one to conclude that the hay actually eaten is representative of the soil treatment. Furthermore, the difference in gains of weight between the two sets of sheep is very small (0.1408 lb. and 0.1644 lb. per head per day), and data are not presented to show that this small difference is significant. Thus, normal variation might well account for these differences, and the greater gains might just as well have been made by either group of the sheep if both had been fed identical hays. In view of these discrepancies, the authors' conclusion that although "there were no differences in the concentrations of calcium or phosphorus in these hays, yet in terms of animal nutrition the presence of lime must have altered the physiology of the lespedeza plants during growth" must be accepted with a great deal of caution. In a later experiment, others (79) did observe that there was an appreciable difference in the botanical composition of their treated and untreated hays, much more grass appearing in the untreated materials.

The variability in biological measurements of the feeding value of forages is demonstrated in the work of Albrecht and Smith (107). In most of their experiments, they use only three animals, whereas a minimum of six to eight is usually considered necessary for this type of work. It is interesting to note, however, that though their measurements sometimes indicate a slight difference in favor of crops from soils treated with superphosphate or superphosphate and limestone, at other times they indicate that the crops from untreated soils appear to be the best. The authors, of course, seek to explain these anomalies, but the critical reader needs a nearer complete presentation of data to gain some appreciation of variability. The presentation of averages, particularly when the differ-

ences are not great and the results do not consistently follow some reasonable trend, is not justified in any biological work. The approach of these investigators is a step forward, however, and the concept of measuring the overall nutritive value of forages as a measure of fertilizer efficiency deserves more attention.

DISCUSSION

It is apparent that studies of the effect of fertilization on the mineral composition of plants have not yet produced sufficient data obtained under a sufficient variety of conditions to permit many generalizations. Thus, most of the recognized factors, such as soils, climate and plant species, that modify the influence of fertilizers, have been studied in little detail. Plant composition is the end result of a number of physiological processes, many of which are controlled by environmental factors whose influences are little understood. Even in carefully controlled nutrient solution culture work, the relationships of cause and effect are not always clear. Under such experimental conditions, however, the consistency of some results indicates a correlation of certain combinations of nutrient supply with certain net effects in the plant. Thus it seems to be generally true that the calcium concentration in the plant is negatively correlated with potassium supply in the nutrient solution. It is interesting to note that there is also a remarkable unanimity of agreement on this relationship as a result of soil experiments.

Although a direct translation of the results obtained in this way to the conditions in any soil is still not advisable in all respects, any interpretation of the composition of plants in terms of a soil's characteristics and the fertilizer applied can be made only by taking into consideration the relationships demonstrated in culture solutions. Peech and Bradfield (95) point out, for example, in their analysis of the calcium-potassium interaction in soils, that plants absorb the greater part of their nutrient cations from the soil solution. The interchange of ions on the solid phases of the soil system renders the whole relationship more complex, however, than is true of the simple solution culture. Thus, Jenny and Ayers (65) have shown that the exchangeability of absorbed potassium is proportional to its percentage saturation of the exchange capacity of the colloid. Furthermore, this relationship is greatly affected by the

nature of the complementary ion. This means that addition of any salt to the soil that would displace one of the absorbed ions from the colloid would upset the existing ratio of ions in the soil solution and consequently influence the uptake of one or more ions by the plant.

The variability of plant composition with fertilization on soils of different kinds and origins is therefore not surprising, for the effects

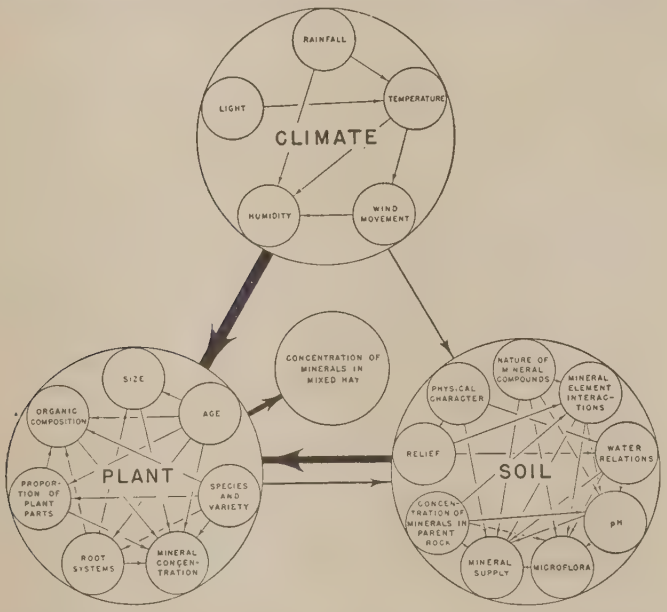

FIG. 1. Factors affecting the mineral composition of a mixed hay. The width of the arrows indicates qualitatively the effects of climate and soil.

of the soil colloidal material and of the soil solution are responsible only in part for these differences in response. Differences in climate, native vegetation and soil parent materials also contribute to these effects. Thus, the Podzolic and Lateritic soils are well-leached acid soils generally quite low in bases and phosphorus, while the Chernozem and Chestnut soils, which are not leached, are high in base constituents and are nearly neutral in reaction. Within

any of these soil groups, moreover, there will be variations in fertility and reaction because of differences particularly in parent material and relief. For example, in the Chernozem soils deficiencies in potassium and phosphorus may exist where the parent materials are very low in these elements. In the rolling and hilly sections of the Kentucky and Tennessee bluegrass regions, erosion removes the acid and leached materials of the soil as rapidly as they are formed, the soil that remains is young and relatively immature, and calcareous material often high in phosphorus lies within the reach of the plant roots.

In Figure 1 an attempt has been made to summarize graphically the known factors that would affect the mineral composition of a mixed hay. These are divided in three parts: (*a*) The discrete elements of climate, (*b*) the chemical, physical and topographic characteristics of soils, and (*c*) the character and development of plants. It is evident that within any one group one must deal with a highly complex and relatively little understood system. Thus, the water relationships in soils are known to affect profoundly the mineral supply of the soil, but the magnitude and nature of these effects are probably greatly modified by other factors, *e.g.*, the physical character of the soil and the nature of the mineral compounds present. Likewise, in studies of plant composition, the factor most commonly ignored is the part played by differences in the proportion of plant parts such as the leaves and stems.

In this scheme (Figure 1) it is intended to stress both the direct and indirect effects of climate on the mineral composition of the plant. The direct effect is probably greater than the indirect effect that is derived by the plant from the soil. It should also be noted that the plant plays its part in the development of the soil and that this in turn has its influence on plant composition. When one considers, therefore, the complexity of the biological system under which we produce our food crops, the variable results obtained by the superimposition of a fertilizer will be recognized as natural. The problem is one that requires detailed study of each of these factors and of its effect on the others, as well as determinations of the overall or net effects as found in each soil as a unit.

Two deficiencies of fundamental importance are generally characteristic of much of the work reported in this review. First, only a few workers have attempted to design experiments that will

measure variability. It is well known that when a single experiment is replicated several times, different results are usually obtained. This is because a number of factors that influence results cannot be controlled. It is almost impossible to repeat any type of biological experiment under practically the same conditions and obtain exactly the same result. Plants growing side by side in the greenhouse and under the most exacting control may not be exactly alike in composition. The necessity for experiments designed to measure the effect of uncontrolled conditions must be recognized before any significant advances can be made in a study of factors affecting plant composition.

Secondly, while the objective of most fertilizer experiments with forage must be the over-all improvement of pasture or hay, the fundamental importance of the effect on single species should not be ignored. Collander (32) has pointed out that single plant species are constantly found to be relatively rich in certain cations, and other species are rich in other cations. Thus, it can be demonstrated repeatedly that applications of lime or nitrogen will have profound effects on the botanical composition of pasture herbage and consequently on the chemical composition of the herbage without regard to that of the single species.

The need for standardizing the nutritive value of plants in terms of animal health is obvious. Certainly any limited number of laboratory determinations is inadequate, and our information does not permit an evaluation of all known factors to be made by this method. Therefore, properly planned animal experiments with crops of known history, accompanied by detailed chemical study of the crops with respect to the nutrients required by animals, should prove very useful in evaluating the effects of soils and fertilization. In undertaking such experiments, however, it must be realized that animal performance is subject to as many variables as is soil fertility and that these variables must be taken into account in planning the experiment and in interpreting the results. There are differences in the nutritive value of rations which are not measurable in terms of growth performance but which require refined physical, biochemical and histological techniques. Two crops may vary widely in the content of a given nutrient and yet the differences may not be evident unless the crops are fed at restricted levels of intake in rations otherwise adequate in all respects. Without con-

trol of the variables involved in animal experimentation, differences in results may be incorrectly attributed to certain known soil and plant differences. For example, marked changes in growth performance may result merely from differences in palatability or from the general unsuitability of the rations for the species in question.

Animal experiments are essential for the evaluation of certain nutritive differences such as those based upon variation in the amino acid make-up of proteins and upon vitamin differences not susceptible to determination by chemical or micro-biological methods. They are useful in searching for undiscovered differences in nutritive value and undiscovered nutritive needs. They also must be the final test of the differences in nutritive value which are measured by chemical or other means.

SUMMARY

From studies of the effect of soil fertilization on the nutritive value and mineral composition of plants, it is possible to make only a few generalized conclusions at this time:

1. The concentration of phosphorus in most species utilized for forage can be increased slightly by use of phosphate fertilizers, particularly where the original phosphorus concentration in the plant is unusually low. There seems to be evidence, however, that the phosphorus concentration in any species may be relatively resistant to change. There is considerable evidence to indicate that the response of phosphorus concentration in the plant to application of phosphates to the soil is dependent to a large degree on the nature of the soil.

2. The effects of nitrogen applied to the soil on the concentration of phosphorus in the plant are inconclusive whether the nitrogen is applied alone or with other fertilizer elements.

3. Any effects of potassium alone or with other elements on the concentration of phosphorus in plants seem to be variable and of little practical significance.

4. Liming the soil seems to depress the concentration of phosphorus in plants under some conditions, while no effect is evident under others. This is probably a soil factor. Liming accompanied by application of phosphates or complete fertilizer mixtures has been found effective under some conditions in increasing the phosphorus concentration in plants.

5. Of several other elements studied, only applications of magnesium to the soil seems to be associated with an increase in the concentration of phosphorus in the plant.

6. In general, liming the soil has resulted in a higher concentration of calcium in the plant, although it is evident that plant species and soil conditions will act to modify this factor measurably.

7. The effect of nitrogen in reducing the percentage of calcium in plants has been confirmed under a number of widely variable soil conditions and in many plant species.

8. Application of calcium as superphosphate has not ordinarily been associated with any change in the calcium concentration in the plant.

9. A reduction in the concentration of calcium in plants has generally followed potash fertilization. No positive correlations of calcium in plants with potassium supply have been reported.

10. There is general agreement that applications of magnesium to the soil result in a lower concentration of calcium in plants, although the differences are seldom of practical importance except where the calcium level is already low.

11. The number of soil experiments in which the micronutrients have been studied are too few to permit any generalizations as to their influence on the general composition of the plant. The data available suggest some important interactions, and these are supported by the work in solution cultures. It is generally true that applications of boron, cobalt, copper and manganese result in greatly increased absorption of these elements by the plant.

12. There is some evidence that liming practices and intensive fertilization under conditions of a limited supply of micronutrients such as boron, manganese, iron and cobalt may further reduce the amount of these elements in the plant.

13. The desirability of correlating the results of animal feeding experiments with plant composition studies is emphasized. Sound and precise animal feeding experiments designed to test the effect of fertilization on the nutritional value of forages are indicated as one of the approaches that will reveal needed information in this field of research.

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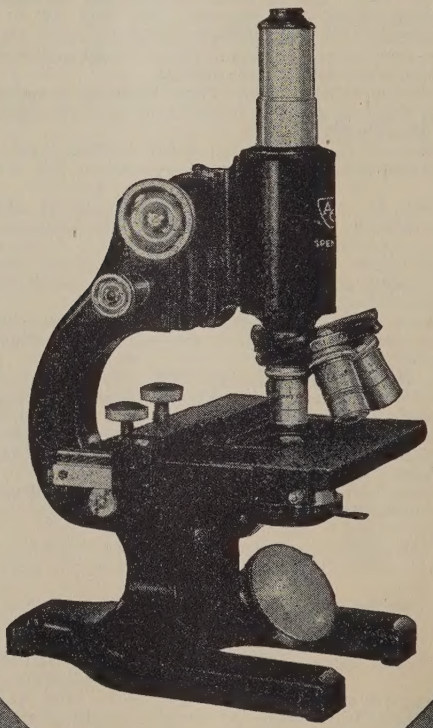

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